A REVIEW OF *SALSUGINUS SECULUS* (PLATYHELMINTHES: MONOGENEA) IN THE WESTERN MOSQUITOFISH (*GAMBUSIA AFFINIS*) FROM TEXAS

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A REVIEW OF *SALSUGINUS SECULUS* (PLATYHELMINTHES: MONOGENEA)

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by

GIZELL VASQUEZ

APPROVED:

Dr. Nicholas J. Negovetich

Dr. Ned E. Strenth

Dr. Loren K. Ammerman

Dr. Jesse Taylor

April 20th, 2016

APPROVED:

Dr. Susan E. Keith
Dean, College of Graduate Studies

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ABSTRACT

Western mosquitofish (*Gambusia affinis*) are cosmopolitan in warmer climates and can serve as a host for a number of parasites. The gill monogeneans from this host are especially interesting because of the very low species diversity that has been reported in the literature. In the United States alone, only a single species, *Salsuginus seculus*, is reported from the gills of fish collected across the country. To assess the validity of a single species on the gills of *G. affinis*, fish were collected from 11 geographically-distant aquatic systems in Texas and surveyed for monogenean infections. The gill monogeneans were then subjected to morphological and molecular analyses. Preliminary analyses suggested that more than one species of monogenean may be infecting the gills of *G. affinis* in Texas. Morphologically, most of the monogeneans resemble *S. seculus*, but parasites from a few locations exhibited anatomical differences that did not conform to the description of *S. seculus*. They are smaller in overall size and differ in their opisthaptor, including the size of the hamuli and the length of the haptoral bars. Sequence analysis on these parasites was performed to determine if consistent patterns of genetic differences exist between specimens collected from the various locations. Based on analysis of a partial 28S rRNA gene region, sufficient support is lacking to distinguish these parasites as different species.

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INTRODUCTION

Western mosquitofish, *Gambusia affinis*, are native to the southern United States and northern Mexico but are now globally distributed due to their use in mosquito control, with the earliest record of interstate transportation in 1905, from Texas to the state of Hawaii (Krumholz, 1948). *Gambusia affinis* has been the subject of many studies (Krumholz, 1948; Pyke, 2005; Lamatsch et al., 2015), but little is known about the monogenean species that infect them.

The class Monogenea is a group within Platyhelminthes that parasitizes aquatic or semi-aquatic animals (Hoffman, 1999). Monogeneans are usually ectoparasitic, living on the surface of their host where they can feed on blood, mucus, and epithelium. Adults attach to their host by the prohaptor, a sucker modification at the anterior end, and by the opisthaptor, a modification of the posterior end that usually contains hooks. There has been a substantial amount of structural adaptation in the opisthaptor of these organisms; this variation is commonly utilized in the taxonomic identification of individual specimens (Hoffman, 1999). Most of the diversity exhibited in the opisthaptor is in its size, shape, and in the number of hooks it contains. Additionally, the diversity exhibited in these attachment structures reflects the host specificity present in this class of parasites. The survival of the worm depends on its effectiveness in attachment, which is ultimately determined by the structure and function of the opisthaptor (Hoffman, 1999).

Monogeneans have a direct, one-host life cycle, thus the dispersal and spread into new aquatic systems of these parasites is highly dependent on their host. Furthermore, their spread to distant locations is inhibited by their limited dispersal ability, but these parasites

can expand their distribution by the movement of infected hosts. Aquatic systems vary in their abiotic conditions throughout the United States, and it is recognized that ectoparasites of fish are susceptible to abiotic fluctuations in their environment (Granath and Esch, 1983; Marcogliese and Esch, 1989). For ectoparasites of fish to become established in a new aquatic system, the survival of both the host and parasite is required. The range of environmental conditions to which monogeneans can adapt and the extent of their intraspecific morphological diversity is unclear. Thus, the regular identification of the same, minute gill monogeneans on fish from geographically and perhaps ecologically different locations is questionable.

Salsuginus seculus (Mizelle and Arcadi, 1945; Murith and Beverley-Burton, 1985) is the common identification for the monogenean species found on the gills of the globally-distributed mosquitofish. Incidentally, *G. affinis* is also the only known host for this species of monogenean (Hoffman, 1999). Specimens of *S. seculus* have been recorded from Texas, California, Louisiana, and the Bahama Islands (Hoffman, 1999); all were identified from the same host species, *G. affinis* The type locality of the western mosquitofish is in Texas from the Rio Medina and Rio Salado (near San Antonio, TX). This species also has a close relative, *Gambusia holbrooki* (the eastern mosquitofish), which is similar in appearance and behavior, but native to the southeastern United States (Robins et al., 1986; Pyke, 2005). Until 1988, *G. affinis* and *G. holbrooki* were considered the same species (Pyke, 2005). The implications of this classification could explain why there is currently no literature describing the gill monogenean species that infect *G. holbrooki*, or why *S. seculus* has only been reported from *G. affinis*. Both species of *Gambusia* are found in Texas (Thomas et al., 2007), and both can hybridize with other members of the genus *Gambusia* (Pyke, 2005). As such,

correct identification of the host from which the monogenean is collected is important for determining the degree of host specificity for *S. seculus*.

Molecular techniques are being increasingly utilized by parasitologists to confirm species identification. They are also being used to assess relationships within the groups of the phylum Platyhelminthes (McManus and Bowles, 1996). Ribosomal DNA sequences in particular have been used to examine the phylogeny of monogenean parasites (Chaudhary et al., 2014). Within the class Monogenea, molecular phylogenetic analysis of 18S and ITS1 ribosomal genes was used to establish *Dactylogyroides longicirrus* as a species (Chiary et al., 2013). The problems present in morphological taxonomy, i.e., phenotypic plasticity, have been observed throughout the animal kingdom (Pfennig et al., 2010). Therefore, there is great potential for a DNA-based identification system to improve the knowledge of the systematics of organisms, especially parasites (Chaudhary et al., 2014).

The current identification of *S. seculus* based on morphology infers that they would possess a similar genetic sequence of conserved regions. However, the unique environmental conditions of the aquatic systems may influence the development of the parasite, such that genetically similar specimens may be morphologically different. Badets et al. (2009) discovered the development of two morphologically distinct phenotypes in the monogenean *Polystoma gallieni* depending on the host physiological stage to which the free-swimming larvae (oncomiracidia) attach. In contrast, morphologically similar parasites may be genetically distinct, thus suggesting different species (Pfenninger and Schwenk, 2007). For example, Hanelt et al. (2015) highlighted the genetic differences among morphologically-identical members of a previously recognized, single species of freshwater nematomorphs based on the cytochrome c oxidase subunit I (COI) gene region. Their analysis identified 8

clades, each harboring a single distinct species. While the morphological identification of *S. seculus* from these distantly located parasites might in fact be true, DNA analyses may resolve the validity of the taxonomic assignment to a single species.

The goal of this study was to investigate the validity of the species *S. seculus* found to infect mosquitofish from various aquatic systems in Texas. The first objective was to genetically confirm the identity of mosquitofish as *G. affinis* in all sampling locations. Then, morphological and molecular analyses was used to assess the identification of *S. seculus* isolated from the fish.

METHODS

Mosquitofish were collected from 11 aquatic systems across Texas (Table I) between the months of April and October 2015. The sites were selected to emphasize (1.) presence of fish and parasite, and (2.) geographic distance between sites. Aquatic system collection sites were from or around the following cities in Texas: San Angelo, Christoval, Knickerbocker, Comstock, Lubbock, Amarillo, Houston, Victoria, Ft. Worth, Sheffield, and San Antonio. Mosquitofish were collected using a dip net.

Female fish were necropsied to inspect for monogenean infection. For each fish, a small sample of muscle tissue was trimmed and preserved in 95% EtOH for DNA analysis. Gills were isolated and inspected under a dissection microscope for monogeneans, and if present, monogeneans were removed by light scraping of the gill filaments using fine insect pins. For molecular analysis, a few of the isolated monogeneans (identified to individual fish) were preserved in 95% EtOH, and the remaining monogeneans were prepared for morphological identification. In total, 114 parasite specimens were isolated from 114 host specimens for DNA analysis, and slides of monogeneans were made from hosts collected across all aquatic systems.

Morphological identification of parasite

Morphological identification of the monogeneans was performed by assessing conformity of their morphological features to the species description of *S. seculus* (Mizelle and Arcadi, 1945). Permanent mounts were prepared by isolating and staining the worms with Gomori Trichrome stain (Titford, 2009), followed by fixation of the parasite in Gray and Wess' medium (Humason, 1979). The trichrome stain permitted viewing of the muscle and connective tissues, while the mounting media cleared the specimens so sclerotized

internal structures could be observed (Thatcher, 2006). Not all structures could be observed using permanent mounts, so an alternative method using live monogeneans was employed. Temporary wet mounts were utilized and attempts were made to record photos and videos of at least three adult monogeneans from each location. Videos and photos were taken with an Olympus DP27-CU microscope digital camera, and measurements were made with the camera control software on the standalone connection kit (DP2-SAL, Olympus Corporation).

Subjective characters from the species description of *S. seculus* were omitted from the analysis. Thus only directly observable and measurable features were analyzed including body length, body width, eyespot number and description, pharynx diameter, haptor length, haptor diameter, ventral anchor length and width of base, dorsal anchor length and width of base, ventral bar length, dorsal bar length, number of haptoral hooks, and junction of haptor and peduncle. All measurements are given in μm and presented as a range. Moreover, the terminology used and characters measured are in accordance with the protocols of Murith and Beverley-Burton (1985).

DNA analysis

DNA from both host and parasite was sequenced to confirm species identification based on phylogenetic analysis. DNA was first extracted from 114 host and 114 parasite tissue samples using a Qiagen blood and tissue kit (Qiagen, Valencia, California). The host and parasite samples matched so that DNA was extracted from those hosts that provided the monogeneans that were ultimately sequenced. Sequence data for *S. seculus* is unavailable. As such, a total of 52 primer pairs were tested in an attempt to obtain both nuclear and mitochondrial sequences for the monogenean parasites (Tables II, III, and IV).

Table I: Collection sites listed by municipality and water system. The coordinates mark the center of the collecting location. The sample size is the number of mosquitofish (*Gambusia*) from which monogeneans were collected from each location.

Collection Site	Water System	Abbreviation	Coordinates	Sample size
San Angelo	Lake Nasworthy	LN	31°23'18.1"N 100°28'34.4"W	11
Knickerbocker	Dove Creek	DC	31°16'38.7"N 100°37'45.5"W	13
Comstock	Devils River	DR	29°42'44.9"N 101°01'11.8"W	13
San Antonio	Medina River	MR	29°47'34.6"N 99°14'52.0"W	13
Christoval	Anson Spring	AS	31°08'06.0"N 100°29'33.8"W	11
Lubbock	Buffalo Springs Lake	BS	33°31'51.0"N 101°42'26.2"W	10
Victoria	Spring Creek	SC	28°50'30.8"N 97°00'41.9"W	11
Houston	Oyster Creek	OC	29°35'20.6"N 95°35'32.0"W	11
Amarillo	Prairie Dog Town Fork Red River	PD	34°50'14.0"N 101°24'58.6"W	10
Sheffield	Pecos River	PR	30°39'38.7"N 101°46'12.8"W	7
Fort Worth	Marys Creek	MC	32°43'12.8"N 97°30'38.3"W	10

These primer pairs were selected from closely related genera. Seventeen of the tested primer pairs targeted COI and Cytochrome b (Cyt*b*) mitochondrial regions, while the remaining primer pairs targeted nuclear ribosomal DNA regions 18S, ITS1, ITS2, and 28S. For the mosquitofish, 14 primer pairs were tested, targeting both mitochondrial and nuclear gene regions.

Amplification of the extracted DNA was performed utilizing a Qiagen Taq PCR core kit (Qiagen, Valencia, California). The primer concentrations were 50μM. Target

amplifications were performed under the following conditions except for the annealing temperature, which varied by primer pair (Tables IV): 3 min of 94°C, followed by 35 cycles of 94°C for 1 min, annealing temperature for 30 sec, and 1 min at 72°C. A final extension step at 72°C for 10 min completed the PCR reaction. Amplification of target DNA regions was assessed by running the PCR products on a 1% agarose gel and visualizing the bands with GelRed (Biotium, Hayward, California). PCR products were purified with a Qiagen PCR purification kit (Qiagen, Valencia, California) prior to sequencing.

A small number of primer pairs generated amplicons suitable for sequencing. The amplicons for the monogeneans included the nuclear 28S ribosomal genes (with ITS2 region). The PCR primers used to amplify the nuclear 28S ribosomal gene region for the monogenean specimens were: Neo28F1(2872) and D2(3395) (Table II and III). Two hyper-variable mitochondrial regions COI and Cyt*b* were successfully amplified for the mosquitofish, but because of constraints associated with cost, only the COI gene was used to confirm the identity of the host. The PCR primers used to amplify the mitochondrial cytochrome c oxidase gene region were: COI_GafF and COI_GafR (Table II and III). Sanger sequencing reactions were performed at the SeqWright Neogenomics center in Houston, TX.

Phylogenetic analyses were conducted on 2 different datasets, 1 of the host and the other of the parasite. Outgroup taxa from GenBank were included in the alignment for comparative purposes and are shown with their accession number on the phylogeny. The host alignment was used in the analysis of *Gambusia* sp. to confirm the identity of the hosts as *G. affinis*. The parasite alignment was used to confirm the species of monogenean infecting the gills. All sequences will be submitted into GenBank.

Phylogenetic analyses were performed by generating individual phylogenetic trees of the selected gene regions for the parasites and hosts in MEGA version 7 (Stecher and Tamura, 2015). Sequences from the forward and reverse primers were aligned using MUSCLE (Edgar, 2004), and a consensus sequence was obtained for each specimen. The consensus sequences were then used in the phylogenetic analyses, which consisted of the MUSCLE method (Edgar, 2004) with default parameters for alignments followed by a model test to identify the best fit model for each group of alignments. Distantly related taxa were used as outgroup comparisons, thus indicating the polarity among the in-group. These taxa were selected by performing individual Blastn searches in MEGA7 of the Buffalo Springs Lake (BS) host 1 sequence for the host phylogeny and of the BS parasite 1 sequence for the parasite phylogeny. Outgroup taxa with low E values and high query coverage, max score, and max identity were selected.

Analysis of the COI gene region dataset for the fish host involved 35 nucleotide sequences and 275 base pairs were analyzed for each sequence. The 35 nucleotide sequences were 25 specimen sequences from this study and the remaining sequences were downloaded from GenBank of 3 *G. affinis*, 5 *G. holbrooki*, 1 *G. punctata* and 1 *G. yucatana*. A maximum likelihood method (ML) based on the Kimura 2-parameter model was used to construct the phylogeny using a starting tree generated by the BioNK algorithm and the topology with superior log-likelihood value was selected. All positions with less than 95% site coverage were eliminated, only allowing fewer than 5% alignment gaps, missing data, and ambiguous bases at any of the positions. The bootstrap method was used with 1000 pseudoreplicates to estimate the reliability of this ML phylogeny.

Analysis of the 28S ribosomal gene region dataset for the monogenean parasites involved 39 nucleotide sequences and 778 base pairs were analyzed for each sequence. The 39 nucleotide sequences were 36 specimen sequences from this study and the remaining sequences were downloaded from GenBank of 1 *Haliotrema eukurodai*, 1 *Cichlidogyrus* sp. and 1 *Dactylogyridae* sp, all which are genera within the order Dactylogyrida. Construction of the parasite phylogeny followed similar methods as those for the fish hosts. However, a maximum likelihood method based on the Kimura 2-parameter model was employed with the rate variation model that allowed some of the sites to be evolutionarily invariable ([+I]). All positions with less than 95% site coverage were eliminated.

Table II: List of forward primers from related genera of *Gambusia* (host) and *Salsuginus* (parasite) screened for use in this study. Annealing temperatures utilized in amplification of these regions are shown in Table IV with primer pair.

Specimen	Region	Forward Sequence	Citation	Name
Parasite	18S partial	TGGTTGATCCTGCCAGT	Dang et al., 2010	18C(708)
Parasite	18S partial	GCAGTTAAAAAGCTCGTAGTTGG	Dang et al., 2010	18SF1(573)
Parasite	18S partial	CGGGGAAAGTATGGTTGC	Dang et al., 2010	18-ITSF1(1117)
Parasite	18S partial	GCTGCGTTCTTCATCGATACTCG	Dang et al., 2010	H7(1369)
Parasite	18S partial	CGCGCAACTTACCCACTCTC	Cone et al., 2010	PBS18SF
Parasite	18S partial	TAGAGGAAGTACAAGTCG	Garcia-Vasquez et al., 2015	ITS1-fm
Parasite	18S partial	TTTCCGTAGGTGAACCT	Zietara et al., 2002	ITS1
Parasite	18S partial	CTATTGGAGGGCAGTCT	Matejusova et al., 2001	V4F
Parasite	5.8S(partial)-28S(partial)	ACCCGCTGAATTTAAGCATA	Dang et al., 2010	Neo28F1(2872)
Parasite	ITS2	CATCGGTCTCTCGAACG	Matejusova et al., 2001	ITS1A
Parasite	ITS1	GTAACAAGGTTTCCGTAGGTG	Matejusova et al., 2001	ITS4.5
Parasite	ITS1(partial),ITS2 (partial)	GTTTCCGTAGGTGAACCT	Zietara et al., 2008	ITS1F
Parasite	ITS2, 28S(partial)	ACCCGCTGAATTTAAGCAT	Dang et al., 2010	C1(2906)
Parasite	28S	ATAGATTTGTGCATGATATACCCAGTG	Cunningham et al., 2000	ITSFR-
Parasite	COI	TTGGATCATAAGCGCATYGGTAT	Zietara et al., 2008	FCox6
Parasite	COI	TAATWGGTGGKTTTGGTAA	Plaisance et al., 2008	COI_Mono_5
Parasite	COI	TAATWGGTGGKTTTGGTAA	Plaisance et al., 2008	COI_Mono_5
Parasite	COI	TGACACGCTTAGATGACATGAAG	Shi et al., 2014	gCOIF
Parasite	COI	TATTATTACCTTCAATGGTGTTAG	Zietara et al., 2008	GarcL
Host	18S	CCTGCGGCTTAATTTGACTC	Raut et al., 2010	Forward 18S
Host	Cyt *b*	ATGGCCAACCTACGAAAAAC	Vidal et al., 2010	CytBF1
Host	Cyt *b*	TGACTTGAARAACCAYCGTTG	Orell et al., 2002	CytbGludgL (L15249)
Host	Cyt *b*	CGAACGTTGATATGAAAAACCATCGTTG	Orell et al., 2002	CytbUnvL(L15242)
Host	Cyt *b*	TGAYWTGAARAACCAYCGTTG	Orell et al., 2002	Cytb4xdgl(L14249)
Host	Cyt *b*	CGAAGCTTGATATGAAAAACCATCGTTG	Lydeard et al., 1995	L14724
Host	Cyt *b*	GTGACTTGAAAAACCACCGTTG	Davis et al., 2006	L15058
Host	COI	TAATTGGTGCCCCCGACATG	Lamatsch et al., 2015	COI_GafF
Host	28S	CCTGTTGAGCTTGACTCTAGTCTG	Wiley et al., 2010	28W
Host	28S	AGCCAATCCTTATCCCGAAGTTACG	Wiley et al., 2010	28MM

Table III: List of reverse primers from related genera of *Gambusia* (host) and *Salsuginus* (parasite) screened for use in this study. Annealing temperatures utilized in amplification of these regions are shown in Table IV with primer pair.

Specimen	Region	Reverse sequence	Citation	Name
Parasite	18S partial	GGTAGTAGCGACGGGCGGTGTG	Dang et al., 2010	18G(1753)
Parasite	18S partial	GGTAGTAGCGACGGGCGGTGTG	Dang et al., 2010	18G(1753)
Parasite	18S partial	GCTTCGATGTTGGGCTARTCTC	Dang et al., 2010	28SR1(2984)
Parasite	18S partial	TGATTTGTCTGGTTTATTCCGAT	Dang et al., 2010	L7(2451)
Parasite	18S partial	ATTCCATGCAAGACTTTTCAGGC	Cone et al., 2010	PBS18SR
Parasite	18S partial	CGCTYGAATCGAGGTCAGGAC	Garcia-Vasquez et al., 2015	ITS2-rm
Parasite	18S partial	GGTAATCACGCTTGAATC	Zietara et al., 2002	ITS2R
Parasite	18S partial	CTTTTCAGGCTTCAAGG	Matejusova et al., 2001	V4R
Parasite	5.8S(partial)-28S(partial)	TGGTCCGTGTTTCAAGAC	Dang et al., 2010	D2(3395)
Parasite	ITS2	TCCTCCGCTTAGTGATA	Matejusova et al., 2001	ITSR3A
Parasite	ITS1	AGCCGAGTGATCCACC	Matejusova et al., 2001	ITS2
Parasite	ITS1(partial),ITS2 (partial)	GGTAATCACGCTTGAATC	Zietara et al., 2008	ITS2R
Parasite	ITS2, 28S(partial)	TGGTCCGTGTTTCAAGAC	Dang et al., 2010	D2(3395)
Parasite	28S	GAGACAAGCATATAACTACTGGCAGGA	Cunningham et al., 2000	18SFR
Parasite	COI	CATTTAATCATGATGCAAAAGG	Zietara et al., 2008	16SR
Parasite	COI	TAATGCATMGGAAAAAAACA	Plaisance et al., 2008	COI_Mono_3
Parasite	COI	ACATAATGAAARTGAGC	Plaisance et al., 2008	COI_Mono_int3
Parasite	COI	ATGTCTACCCTAGAGCATGAAAGT	Shi et al., 2014	gCOIR
Parasite	COI	CATAATGAAAATGTGCTACCACAA	Zietara et al., 2008	GarcH
Host	18S	AACTAAGAACGGCCATGCAC	Raut et al., 2010	Reverse 18S
Host	Cyt *b*	GGGTAGRACATAACCTACGAAG	Vidal et al., 2010	CytBR1
Host	Cyt *b*	CTCCAGTCTTCGRCTTACAAG	Orell et al., 2002	CytbThrdgH(H16465)
Host	Cyt *b*	ATCTTCGGTTTACAAGACCGGTG	Orell et al., 2002	CytbUnvH(H16458)
Host	Cyt *b*	TGRVNCTGAGCTACTASTGC	Orell et al., 2002	Cytb4xdgH(H16435)
Host	Cyt *b*	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA	Lydeard et al., 1995	H15149
Host	Cyt *b*	AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA	Davis et al., 2006	H15149
Host	COI	GGAGGACAGCTGTAATTAGGACTGCTCAC	Lamatsch et al., 2015	COI_GafR
Host	28S	GTGAATTCTGTTCACAATGATAGGAAGAGCC	Wiley et al., 2010	28X
Host	28S	GTCTTGAAACACGGACCAAGGAGTCT	Wiley et al., 2010	28DD

Table IV: List of pairs of primers tested in the amplification process of *Gambusia* (host) or *Salsuginus* (parasite) with the annealing temperature (T_n) used. Sequences of the primers are found in Table II and III.

Region	Specimen	Forward	Reverse	Tn
Nuclear	Parasite	18C(708)	18G(1753)	50 C
Nuclear	Parasite	18SF1(573)	18G(1753)	60 C
Nuclear	Parasite	18-ITSF1(1117)	28SR1(2984)	54 C
Nuclear	Parasite	H7(1369)	L7(2451)	60 C
Nuclear	Parasite	PBS18SF	PBS18SR	60 C
Nuclear	Parasite	ITS1-fm	ITS2-rm	50 C
Nuclear	Parasite	ITS1	ITS2R	50 C
Nuclear	Parasite	V4F	V4R	50 C
Nuclear	Parasite	Neo28F1(2872)	D2(3395)	50 C
Nuclear	Parasite	ITS1A	ITSR3A	50 C
Nuclear	Parasite	ITS4.5	ITS2	50 C
Nuclear	Parasite	ITS1F	ITS2R	50 C
Nuclear	Parasite	C1(2906)	D2(3395)	50 C
Nuclear	Parasite	ITSFR-	18SFR	60 C
Mitochondrial	Parasite	FCox6	16SR	50 C
Mitochondrial	Parasite	COI_Mono_5	COI_Mono_3	50 C
Mitochondrial	Parasite	COI_Mono_5	COI_Mono_int3	50 C
Mitochondrial	Parasite	gCOIF	gCOIR	60 C
Mitochondrial	Parasite	GarcL	GarcH	60 C
Nuclear	Parasite	18SF1(573)	D2(3395)	50 C
Nuclear	Parasite	18SF1(573)	28SR1(2984)	50 C
Nuclear	Parasite	18SF1(573)	L7	50 C
Nuclear	Parasite	18SF1(573)	PBS18SR	50 C
Nuclear	Parasite	18SF1(573)	18SFR	50 C
Nuclear	Parasite	18SF1(573)	ITS2-rm	50 C
Nuclear	Parasite	18SF1(573)	ITS2R	50 C
Nuclear	Parasite	18SF1(573)	ITS2	50 C
Nuclear	Parasite	18SF1(573)	ITSR3A	50 C
Nuclear	Parasite	18SF1(573)	V4R	50 C
Nuclear	Parasite	18C(708)	D2(3395)	50 C
Nuclear	Parasite	18SF1(573)	D2(3395)	50 C
Nuclear	Parasite	H7(1369)	D2(3395)	50 C

continued.

Region	Specimen	Forward	Reverse	Tn
Nuclear	Parasite	PBS18SF	D2(3395)	50 C
Nuclear	Parasite	ITS1-fm	D2(3395)	50 C
Nuclear	Parasite	ITS4.5	D2(3395)	50 C
Nuclear	Parasite	ITS1	D2(3395)	50 C
Nuclear	Parasite	ITS1A	D2(3395)	50 C
Nuclear	Parasite	ITSFR	D2(3395)	50 C
Nuclear	Parasite	ITS1F	D2(3395)	50 C
Nuclear	Parasite	V4F	D2(3395)	50 C
Mitochondrial	Parasite	gCOIF	GarcH	60 C
Mitochondrial	Parasite	gCOIF	16SR	60 C
Mitochondrial	Parasite	gCOIF	COI_Mono_3	60 C
Mitochondrial	Parasite	gCOIF	COI_Mono_int3	60 C
Mitochondrial	Parasite	FCOX6	GarcH	60 C
Mitochondrial	Parasite	FCOX7	COI_Mono_3	60 C
Mitochondrial	Parasite	FCOX8	COI_Mono_int3	60 C
Mitochondrial	Parasite	FCOX9	gCOIR	60 C
Mitochondrial	Parasite	COI_Mono_5	gCOIR	60 C
Mitochondrial	Parasite	GarcL	gCOIR	60 C
Mitochondrial	Parasite	COI_Mono_5	16SR	50 C
Mitochondrial	Parasite	GarcL	16SR	60 C
Nuclear	Host	Forward 18S	Reverse 18S	60 C
Mitochondrial	Host	CytBF1	CytBR1	58 C
Mitochondrial	Host	CytbGludgL (L15249)	CytbThrdgH(H16465)	72 C
Mitochondrial	Host	CytbUnvL(L15242)	CytbUnvH(H16458)	58 C
Mitochondrial	Host	Cytb4xdgl(L14249)	Cytb4xdgH(H16435)	58 C
Mitochondrial	Host	L14724	H15149	72 C
Mitochondrial	Host	L15058	H15149	58 C
Mitochondrial	Host	COI_GafF	COI_GafR	58 C
Nuclear	Host	28W	28X	72 C
Nuclear	Host	28MM	28DD	72 C
Nuclear	Host	Forward 18S	28X	60 C
Nuclear	Host	Forward 18S	28DD	60 C
Nuclear	Host	28W	Reverse 18S	60 C
Nuclear	Host	28MM	Reverse 18S	60 C

RESULTS

Morphological identification of parasite

A total of 14 characters were directly observed or measured on the monogeneans (Table V). Morphological analyses of live specimens for Springs Creek (Victoria, TX) and Anson Springs (Christoval, TX) could not be performed due to the lack of infection on the host at the time of photo and video recording. Additionally, morphological analyses are lacking for live specimens of the Pecos River (Sheffield, TX) due to the mass death of fish hosts in the lab before these analyses could be performed. Differences were found in all 8 locations in which morphological analyses were possible (Table V). Some of the measurements were larger and others were shorter than depicted in the species description of *S. seculus* (Mizelle and Arcadi, 1945). Moreover, the dorsal and ventral bars varied in size for some locations, which deviated from the species description.

DNA analysis

Identification of *G. affinis* as the host species for all 11 locations was confirmed by the COI marker using a maximum likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood was selected (Fig. 1). The analysis involved 33 nucleotide sequences, and in the final dataset, 275 base pairs were analyzed for each sequence. With 94% bootstrap support, the fish host samples of this study fall within a single clade, within this clade are also the 3 sequences of *G. affinis* downloaded from GenBank. The sequences were further analyzed by computing pairwise distances with a K2 parameter model in MEGA7. All fish host sequences of this study exhibited 99% genetic similarity

within the cluster of *G. affinis* individuals and a 97% genetic similarity was calculated for their closest relative in GenBank, *G. holbrooki*.

For the monogeneans, sequence data for the partial 28S ribosomal gene was obtained for all 11 aquatic systems. A maximum likelihood phylogeny generated 100% bootstrap support for a single clade containing all the monogeneans from this study. The analysis involved 39 nucleotide sequences, and in the final dataset, 778 base positions were analyzed for each sequence. As such, all monogeneans appear to be the same species, *S. seculus* (Fig. 2). The sequences were further analyzed by computing pairwise distances with a K2 parameter model in MEGA7. All monogenean parasite sequences of this study exhibited 98% genetic similarity. In comparison, *Haliotrema eukuroda*, exhibited a 71% genetic similarity, while the outgroup genus *Cichlidogyrus* sp., exhibited a 70% genetic similarity and the outgroup genus *Dactylogyridae* sp., shared a 69% genetic similarity with the monogeneans of this study.

Table V: Characteristics analyzed and measurement ranges (in μm) calculated for the monogenean specimens, *Salsuginus seculus* (N=3 from each site). The first column of ranges is from the species description of *S. seculus* by Mizelle and Arcadi (1945).

	Whittier, CA **San Gabriel River**	Lubbock **Buffalo Springs Lake**	Amarillo **Prairie Dogtown F.R.R.**	Fort Worth **Marys Creek**	San Antonio **Medina River**	Houston **Oyster Creek**	Knicker-bocker **Dove Creek**	Comstock **Devils River**	San Angelo **Lake Nasworthy**
Feature									
Body Length	243-353	152-227	170-223	181-183	217-234	215-227	210-338	162-303	280-288
Body Width	65-108	58.1-84.3	57.8-85.2	57.3-60.2	60.9-75.0	61.3-73.9	51.5-76.0	3.70-6.76	6.42-8.8
Eyespots*	4, Yes	4, Yes	>4 Yes	4, No	2-4, Yes	2, Yes	4->4, No	2, Yes	>4, No
Pharynx diameter	20-36	14.0-16.0	14.3-18.5	14.7-15.3	16.1-22.7	16.4-20.0	14.7-17.5	14.2-18.4	16.4-20.0
Haptor Length	34-52	27.4-31.)	26.0-43.3	23.0-29.65	28.1-43.2	32.1-42.4	29.6-33.0	25.1-32.0	22.4-36.6
Haptor diameter	52-77	53.0-67.6	48.5-79.1	52.3-58.8	57.9-73.7	60.6-73.1	55.6-65.2	46.4-70.0	58.1-62.2
Haptor and peduncle†	Joined	Separate	Separate	Separate	Separate	Separate	Separate	Separate	Separate
Ventral anchor length	18-22	16.4-21.5	18.2-20.0	20.9-21.1	17.4-17.5	17.1-18.7	18.2-19.2	17.4-23.0	16.0-21.1
Ventral anchor width of base	10-5	11.0-14.7	10.3-11.3	11.3-13.2	12.3-14.8	13.4-14.0	10.6-14.2	7.56-11.6	10.81-12.40
Dorsal anchor length	16-20	14.2-18.2	16.0-18.8	17.6-18.3	16.0-16.3	18.2-18.9	16.9-21.2	19.0-20.6	14.7-19.1
Dorsal anchor width of base	9-14	9.70-11.1	10.0-10.1	9.80-11.8	11.2-12.9	11.5-12.7	9.95-10.1	8.91-11.3	11.6-11.8
Bars length‡	Yes	No	No	No	Yes	No	No	No	No
Ventral bar length	20-25	18.95-23.6	20.0-24.4	20.8-22.8	22.8-23.3	28.3-28.9	22.6-23.8	23.2	19.6-21.3
Dorsal bar length	20-24	17.7-22.4	20.3-21.3	18.7-19.9	23.3-23.7	21.3-22.7	19.5-24.0	22.8	20.6-21.6
# of Hooklets	14	12	Maybe >14	16	16	12	12-16	12-14	14

*Eyespot number and description: if the anterior pair was melanistic then it is marked as "yes".

†Haptor, peduncle: If haptor and peducle had distinct separation then it is marked as "separate".

‡Bars length: if the ventral and dorsal transverse bars were approximately equal in length (<1μm in difference) then it is marked as "yes".

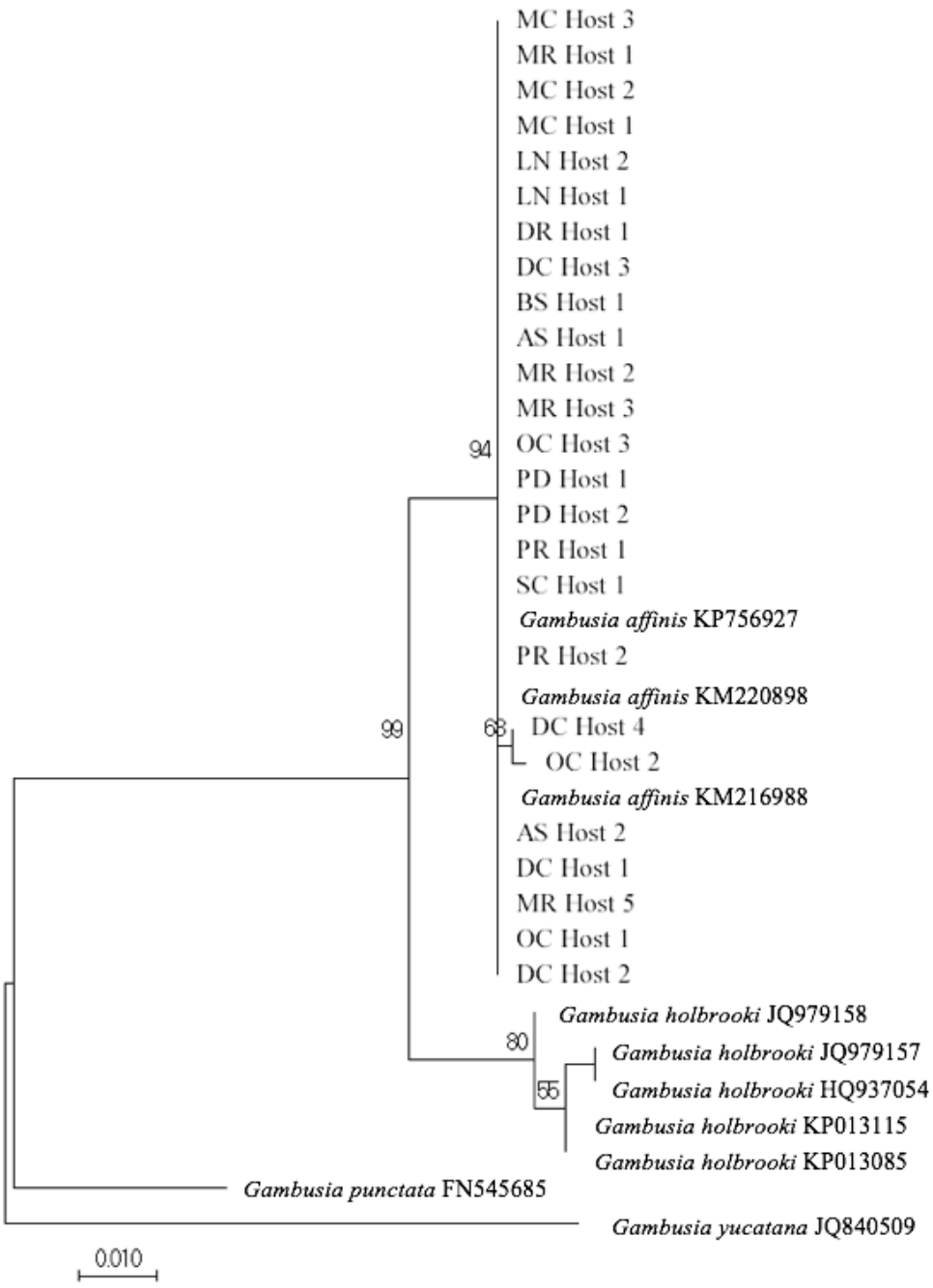

Figure 1: Maximum likelihood phylogeny of specimens identified as *Gambusia affinis,* using the COI mitochondrial gene. Values on branches are the percentage of trees from bootstrap analysis in which the associated taxa clustered together. The Kimura 2-parameter maximum likelihood model (Kimura, 1980) was used. The tree with the highest log likelihood (-587.1729) is shown and drawn to scale, with branch lengths measured in the number of substitutions per site. The abbreviations are representative of the varied aquatic system from which the host and parasite was collected (Table I).

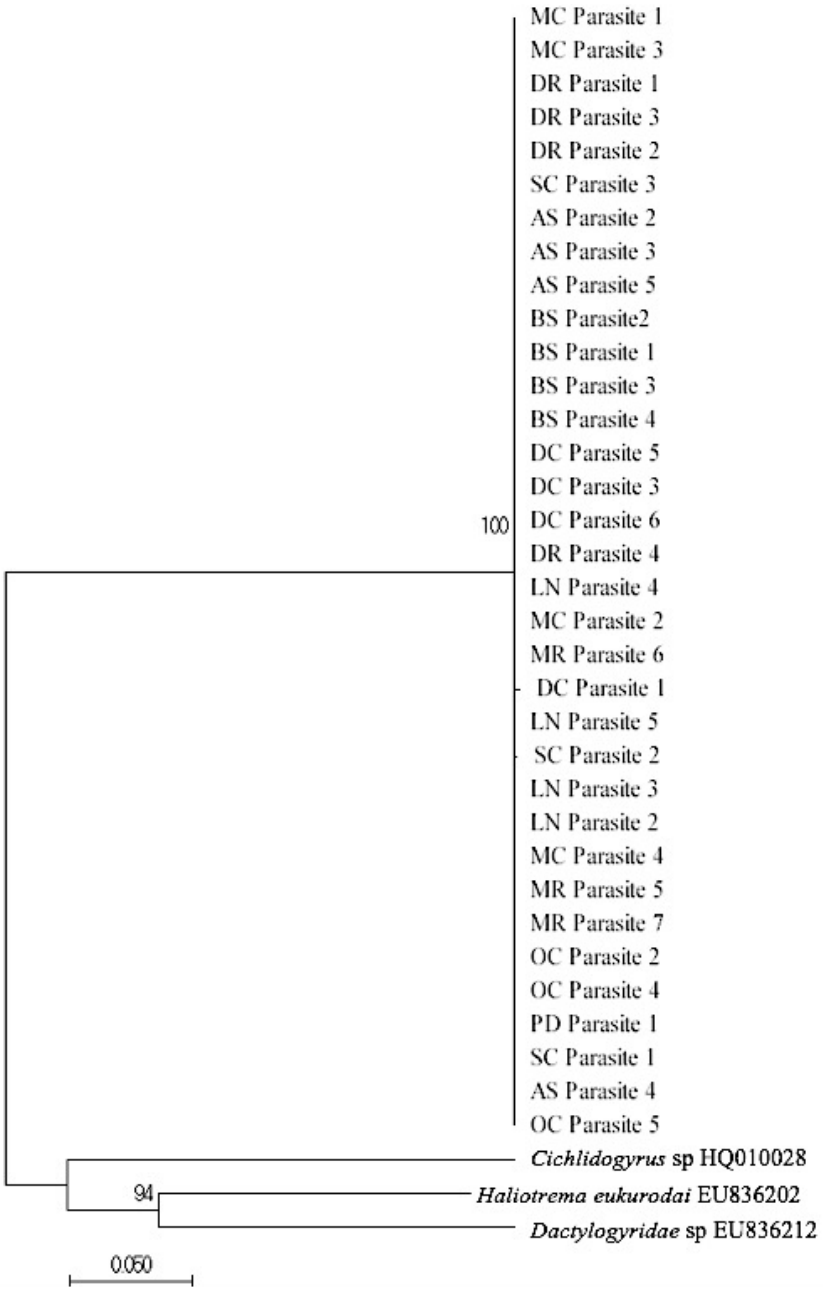

Figure 2: Maximum likelihood phylogeny of specimens identified as *Salsuginus seculus* using the 28S ribosomal gene. Values on branches are the percentage of trees from bootstrap analysis in which the associated taxa clustered together. The Kimura 2-parameter maximum likelihood model (Kimura, 1980) was used, which allowed for some sites to be evolutionarily invariable ([+I], 30.4627% sites). The tree with the highest log likelihood (-2648.8019) is shown and drawn to scale, with branch lengths measured in the number of substitutions per site. The abbreviations are representative of the varied aquatic system from which the host and parasite was collected (Table I).

DISCUSSION

The goal of this study was to determine whether the monogeneans collected from geographically-distant aquatic systems conformed to the morphological species description of *S. seculus* (Mizelle and Arcadi, 1945). This description included a statement that the parasite infected *G. affinis*. By utilizing molecular data, *G. affinis* was confirmed as the host species in this study (Fig. 1). The morphological variation observed in the monogeneans, therefore, cannot be attributed to infection on more than one host species. In general, the monogeneans did conform to the morphological description of the genus *Salsuginus* in that they were found on freshwater fish and possessed four anchors, a haptor with 2 transverse bars, a curved, tubular cirrus with inflated base, and a variable accessory piece not articulated basally (Hoffman, 1999). Yet, specimens from some locations deviated from the species description. Notable differences include the number of hooklets present on the haptor, number and melanization of the eyespots, and length of both the ventral and dorsal anchors. Length among the ventral and dorsal transverse bars also varied from the expectation that they be approximately equal in length (Table V). These morphological differences represent more variation than described by Mizelle and Arcadi (1945) for *S. seculus*.

Species should exhibit genetic divergence from other species (Pfennig et al., 2010). Specifically, phylogenetic analysis of the partial 28S ribosomal gene region should reveal the presence of more than one clade if the molecular data matches the morphological data. However, the data generated a single clade with 100% bootstrap support. Thus, the sequence data suggest the monogeneans are the same species. Comparisons among the sequences further support the existence of a single species, with a 98-99% genetic similarity among the monogeneans of this study. Comparatively, the outgroup taxa clustered into a single clade

with a 69-71 % genetic similarity to its sister clade, the monogeneans from this study. Alternatively, the 28S gene region may be too conserved in identifying various species within *Salsuginus* as is the case for several other organisms within the animal kingdom. A more variable region, such as the nuclear 18S or ITS1 region (Chiary et al., 2013), or mitochondrial COI may be required to better understand the taxonomic classification of phenotypically plastic species.

The monogeneans collected from the various locations in Texas appear morphologically variable but are genetically similar. This morphological variation could arise from several sources, the first of which is specimen preparation. The species description of *S. seculus* was based on examination of 20 specimens fixed in glycerine jelly (Mizelle and Arcadi, 1945). The methods of slide preparation were very detailed and specifically stated great care be taken to avoid altering the specimens by excessive cover-slip pressure (Mizelle and Arcadi, 1945). Overall size and shape of the worm may be altered if the coverslip is too large or heavy. For example, worms under a coverslip may be flattened and produce larger measurements of length and width than specimens measured without coverslip pressure. Additionally, measurements could vary based on the fixative and mounting medium (Murith and Beverly-Burton, 1985). While methodology of specimen preparation varied in this study compared to Mizelle and Arcadi (1945), the variation in technique should not alter the shape, size, or number of the sclerotized structures. This has led to *Salsuginus* sp. systematics relying heavily on sizes of hamuli and shape of the accessory piece (Mizelle and Arcadi, 1945; Murith and Beverley-Burton, 1985). As such, morphological differences, especially in the lengths of the dorsal and ventral bars, would support >1 monogenean species on the gills of *G. affinis*.

The problems present in morphological taxonomy have been observed previously in Monogenea. For example, morphometric analysis identified 2 morphotypes of *S. bermudae* infecting both *Fundulus bermudae* and *G. affinis* in Bermuda (Rand and Wiles, 1987). Each morphotype was specific to the lake from which the fish were collected and differed in the shape of the sclerotized copulatory apparatus and accessory piece. The authors suggested that hydrological differences between the lakes possibly contributed to the variation in the morphotypes (Rand and Wiles, 1987). Morphological variation also has been observed in several other studies of *Salsuginus* sp. (Murith and Beverley-Burton, 1985; Janovy et al., 1989; Ferdig et al., 1993; Mendoza-Franco and Vidal-Martinez, 2001). Thus, phenotypic plasticity may obfuscate species identification based only on morphological characters.

Phenotypic plasticity in other species of the Monogenea has also been previously reported. For example, a molecularly confirmed species of *Ligophorus* exhibits variation in shape and size of haptoral anchors attributable to genetic variation, ontogenetic change, and plastic responses to environmental factors (Rodriguez-Gonzalez et al., 2015).

This study generated the first genetic sequences of *S. seculus* and provided molecular support for a single species of monogenean on the gills of *G. affinis* from 11 locations in Texas. Like other species within the genus, *S. seculus* exhibits morphological variation, and this study demonstrates more variability than initially reported by Mizelle and Arcadi (1945). The underlying cause of the phenotypic plasticity is unclear, but it may be due to environmental variation of the aquatic systems (Perry and Laurent, 1993). This environmental variability, either directly or indirectly (via effects on the host), could ultimately stimulate specific morphological adaptations of the parasite to optimize exploitation of the host (Bush, 2009).

WORKS CITED

Badets, M., J. Boissier, P. Bremond, and O. Verneau. 2009. *Polystoma gallieni*: experimental evidence for chemical cues for developmental plasticity. Experimental Parasitology **121**: 163-166.

Baum, D. A., and S. D. Smith. 2013. Tree thinking: an introduction to phylogenetic Biology. Roberts and Company Publishers, Inc., Greenwood Village, Colorado, 176 p.

Buchmann, K., T. Lindestrom, and J. Bresciani. 2004. Interactive associations between fish, hosts and monogeneans. Host-Parasite Interactions **55**: 165-189.

Bush, S. E. 2009. Does behavioral flexibility facilitate host switching by parasites? Functional Ecology **23**: 578-586.

Cantarore, P., M. Robetim, G. Pesole, A. Ludovico, F. Milella, M. N. Gadaleta, and C. Saccone. 1994. Evolutionary analysis of cytochrome b sequences in some Perciformes: evidence for a slower rate of evolution than in mammals. Journal of Molecular Evolution **39**: 589-597.

Chaudhary, A., C. Verma, and H. S. Singh. 2014. Ribosomal DNA as molecular markers and their applications in the identification of fish parasites (Platyhelminthes: Monogenea) from India. Asian Pacific Journal of Tropical Diseases **4**: 82-84.

Chiary, H. R., A. Chaudhary, and H.S. Singh. 2013. Phylogenetic analysis of the *Dactylogyroides longicirrus* (Monogenea: Dactylogyridae) based on 18S and ITS1 ribosomal genes. Bioinformation **9**: 250-254.

Cone, D., C. Abbott, S. Gilmore, and M. Burt. 2010. A new genus and species of gyrodactylid (Monogenea) from silver hake, *Merluccius bilinearis*, in the Bay of Fundy, New Brunswick, Canada. Journal of Parasitology **96**: 681-684.

Courtenay Jr., W. R., and G. K. Meffe. 1989. Small fishes in strange places: a review of introduced poeciliids. Ecology and Evolution of live-bearing fishes (Poeciliidae). G. K. Meffe, and F. F. Snelson Jr. (eds.). Prentice Hall, Englewood Cliffs, NJ, p. 319-331.

Cunningham, C. O., H. Aliesky, and C. H. Collins. 2000. Sequence and secondary structure variation in the *Gyrodactylus* (Platyhelminthes: Monogenea) ribosomal RNA gene array. Journal of Parasitology **86**: 567-576.

Dang, B. T., A. Levsen, C. Schander, and G. A. Bristow. 2010. Some *Haliotrema* (Monogenea: Dactylogyridae) from cultured grouper (*Epinephelus* spp.) with emphasis on the phylogenetic position of *Haliotrema cromileptis*. Journal of Parasitology **96**: 30-39

Davis, S. K., A. A. Echelle, and R. A. Van Den Bussche. 2006. Lack of cytonuclear genetic introgression despite long-term hybridization and backcrossing between two Poeciliid fishes (*Gambusia heterochir* and *G. affinis*). Copeia **2006**: 351-359.

Edgar, R. C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics **5:** 113.

Ferdig, M. T., M. A. McDowell, J. Janovy Jr., and R. E. Clopton. 1993. Patterns of morphological variation of *Salsuginus yutanensis* (Monogenea: Ancryocephalidae) over space and time. Journal of Parasitology **79**: 744-750.

Garcia-Vasquez, A., U. Razo-Mendivil, and M. Rubio-Godoy. 2015. Morphological and molecular description of eight new species of *Gyrodactylus* von Nordmann, 1832 (Platyhelminthes: Monogenea) from poeciliid fishes, collected in their natural distribution range in the Gulf of Mexico slope, Mexico. Parasitology Research **114**: 3337-3355.

Granath, W., and G. W. Esch. 1983. Temperature and other factors that regulate the composition and infrapopulation densities of B*othriocephalus acheilognathi* (Cestoda) in *Gambusia affinis* (Pisces). Journal of Parasitology **69**: 1116-1124.

Hanelt, B., A. Schmidt-Rhaesa, and M. G. Bolek. 2015. Cryptic species of hairworm parasites revealed by molecular data and crowdsourcing of specimen collections. Molecular Phylogenetics and Evolution **82**: 211-218.

Heino, J. 2001. Regional gradient analysis of freshwater biota: Do similar biogeographic patterns exist among multiple taxonomic groups? Journal of Biogeography **28:** 69–76.

Hoffman, G. L. 1999. Parasites of North American freshwater fishes, 2nd ed. Comstock Publishing Associates, Ithaca, New York, 539 p.

Humason, G. L. 1979. Animal tissue techniques, 4th ed. W. H. Freeman and Co., San Francisco, California, 661 p.

Janovy Jr., J., T. R. Runkhe, and T. A. Wheeler. 1989. *Salsuginus thalkeni* n. sp. (Monogenea: Ancyrocephalidae) from *Fundulus zebrinu*s in the South Platte River of Nebraska. Journal of Parasitology **75**: 344-347.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution **16**: 111-120.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edward, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Science **88**: 6196-6200.

Krumholz, L. A. 1948. Reproduction in the western mosquitofish *G. affinis affinis* (Baird & Girard), and its use in mosquito control. Ecological Monographs **18**: 1-43.

Lamatsch, D. K., S. Adolfsson, A. M. Senior, G. Christiansen, M. Pichler, Y. Ozaki, L. Smeds, M. Schartl, and S. Nakagawa. 2015. A transcriptome derived female-specific marker from the invasive western mosquitofish (*Gambusia affinis*). PLoS ONE **10**: 1371.

Lydeard, C., M. C. Wooten, and A. Meyer. 1995. Cytochrome b sequence variation and a molecular phylogeny of the live-bearing fish genus *Gambusia* (Cyprinodontiformes: Poeciliidae). Canadian Journal of Zoology **73**: 213-227.

Marcogliese, D. J., and G. W. Esch. 1989. Alterations in seasonal dynamics of *Bothriocephalus acheilognathi* in a North Carolina cooling reservoir over a seven-year period. Journal of Parasitology **75**: 378-382.

Matejusova, I., M. Gelnar, A. J. A. McBeath, C. M. Collins, and C. O. Cunningham. 2001. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). International Journal for Parasitology **31**: 738-745.

Mizelle, J. D., and J. A. Arcadi. 1945. Studies on monogenetic trematodes. XIII. *Urocleidus seculus*, a new species of Tetraonchinae from the viviparous top minnow, *Gambusia affinis affinis* (Baird and Girard). Transaction of the American Microscopical Society **64**: 293-296.

McManus, D. P., and J. Bowles. 1996. Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. International Journal for Parasitology **26**: 687-704.

Mendoza-Franco, E. F., and V. M. Vidal-Martinez. 2001. *Salsuginus neotropicalis* n. sp. (Monogenea: Ancyrocephalidae) from the pike killfish *Belonesox belizanus* (Atheriniformes: Poeciliidae) from southeastern Mexico. Systematic Parasitology **48**: 41-45.

Murith, D., and M. Beverley-Burton. 1985. *Salsuginus* Beverley-Burton 1984 (Monogenea: Ancyrocephalidae) from Cyprinodontoidei (Atheriniformes) in North America with descriptions of *Salsuginus angularis* (Mueller, 1934) Beverley-Burton, 1984 from *Fundulus diaphanus* and *Salsuginus heterocliti* n. sp. from *F. heteroclitus*. Canadian Journal of Zoology **63**: 703-714.

Orrell, T. M., K. E. Carpenter, J. A. Musick, and J. E. Graves. 2002. Phylogenetic and biogeographic analysis of the Sparidae (Perciformes: Percoidei) from cytochrome b sequences. Copeia **2002**: 618-631.

Palumbi, S., A. Martin, S. Romano, W. O. McMillan, L. Stice, and G. Grabowski. 1991. The simple fools guide to PCR. Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Pariselle, A., W. A. Boeger, J. Snoeks, C. F. Bilong, S. Morand, and M.P.M. Vanhove. 2010. The Monogenean parasite fauna of cichlids: A potential tool for host biogeography. International Journal of Evolutionary Biology **2011**: 4061.

Perry, S. F., and P. Laurent. 1993. Environmental effects on fish gill structure and function. Fish Ecophysiology **9**: 231-264.

Pfenninger, M., and K. Schwenk. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evolutionary Biology **7**: 121.

Pfennig, D., M. A. Wund, E. C. Snell-Rood, T. Cruickshank, C. D. Schlichting and A. P. Moczek. 2010. Review of phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology and Evolution **25**: 459-467.

Plaisance, L., V. Rousset, S. Morand, and D. T. J. Littlewood. 2008. Colonization of Pacific islands by parasites of low dispersal ability: Phylogeography of two monogenean species parasitizing butterflyfishes in the South Pacific Ocean. Journal of Biogeography **35**:76-87.

Pyke, G. H. 2005. A review of the biology of *Gambusia affinis* and *G. holbrooki*. Reviews in Fish Biology and Fisheries **15**: 339-365.

Rand, T. G., and M. Wiles. 1987. *Salsuginus bermudae* sp. n. (Monogenea: Ancyrocephalidae) from *Fundulus bermudae* Gunther and *Gambusia affinis* (Baird and Girard) in Bermuda. Canadian Journal of Zoology **65**: 1847-1852.

Raut, S. A., and A. A. Robert. 2010. Triclosan has endocrine-disrupting effects in male western mosquitofish, *Gambusia affinis*. Environmental Toxicology and Chemistry **29**: 1287-1291.

Reece, J. B., L. A. Urry, M. L. Cain, S. A. Wasserman, P. V. Minorsky, and R. B. Jackson. 2013. Evolution. Campbell Biology, 10th ed. Benjamin Cummings, San Francisco, California.

Reed, P., R. Francis-Floyd, R. Klinger, and D. Petty. 2012. Monogenean parasites of fish. The Institute of food and agricultural sciences, University of Florida.

Robins, C. R., G. C. Ray, and J. Douglas. 1986. A Field Guide to Atlantic Coast Fishes. The Peterson Field Guide Series. Houghton Mifflin Co., Boston, Massachusetts, 354p.

Rodriguez-Gonzalez, A., Miguez-Lozano, R., Llopis-Belenguer, C., and J. A. Balbuena. 2015. Phenotypic plasticity in haptoral structures of *Ligophorus cephalic* (Monogenea: Dactylogyridae) on the flathead mullet (*Mugil cephalus*): a geometric morphometric approach. International Journal for Parasitology **45**: 295-303.

Shi, S. F., M. Li, S. Yan, M. Wang, C. P. Yang, Z. R. Lun, C. L. Brown, and T. B. Yang. 2014. Phylogeography and demographic history of *Gotocotyla sawara* (Monogenea: Gotocotylidae) on Japanese Spanish mackerel (*Scomberomorus niphonius*) along the coast of China. Journal of Parasitology **100**: 85-92.

Stecher, K. S., and K. Tamura. 2015. MEGA7: Molecular Evolutionary genetic analysis version 7.0 for bigger datasets. Molecular Biology and Evolution.

Stefan, H. G., M. Hondzo, X. Fang, J. G. Eaton, and J. H. McCormick. 1996. Simulated long-term temperature and dissolved oxygen characteristics of lakes in the north-central United States and associated fish habitat limits. The American Society of Limnology and Oceanography **41**: 1124-1135.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Journal of Molecular Biology and Evolution **10**: 2731-2739.

Thatcher, V. E. 2006. Monogenoidea. Aquatic biodiversity in Latin America: Amazon fish parasites. Sofia, Bulgaria: Pensoft.

Thomas, C., T. H. Bonner, B. G. Whiteside, A. Sansom, and F. Gelwick. 2007. Freshwater fishes of Texas. A&M nature guides. Texas A&M University Press, College Station, Texas.

Titford, M. 2009. Progress in the development of microscopical techniques for diagnostic pathology. The Journal of Histotechnology **32**: 9-19.

Valenti, T. W., J. M. Taylor, J. A. Back, R. S. King, and B. W. Brooks. 2011. Influence of drought and total phosphorus on diel pH in wadeable streams: Implications for ecological risk assessment of ionizable contaminants. Integrated Environmental Assessment and Management **7**: 636-647.

Vidal, O., E. Garcia-Berthou, P. A. Tedesco, and J. Garcia-Marin. 2010. Origin and genetic diversity of mosquitofish (*Gambusia holbrooki*) introduced to Europe. Biological Invasions **12**: 841-851.

Wheeler, T.A, and L. A. Chisholm. 1994. Monogenea vs Monogenoidea, the case for stability in nomenclature. Systematic Parasitology **30**: 159-164.

Williams, H., and A. Jones. 1994. Parasitic worms of fish. Taylor & Francis, London, 593 p.

Wiley, E. O., G. D. Johnson, and W. W. Dimmick. 2000. The interrelationships of Acanthomorph fishes: A total evidence approach using molecular and morphological data. Biochemical Systematics and Ecology **28**: 319-350.

Wooten, M. C., K. T. Scribner, and M. H. Smith. 1988. Genetic variability and systematics of *Gambusia* in the southeastern United States. Copeia **1988**: 283-289.

Zietara, M. S., J. Kuusela, A. Veselov, and J. Lumme. 2008. Molecular faunistics of accidental infections of *Gyrodactylus* Nordmann, 1832 (Monogenea) parasitic on salmon *Salmo salar* L. and brown trout *Salmo trutta* L. in NW Russia. Systematic Parasitology **69**: 123-135.

Zietara, M. S., and J. Lumme. 2002. Speciation by host switch and adaptive radiation in a fish parasite genus *Gyrodactylus* (Monogenea, Gyrodactylidae). Evolution **56**: 2445-2458.

VITA

Gizell Vasquez, was born October 6, 1990 to Israel Vasquez and Lizette Posada of Muleshoe, TX. She attended Angelo State University from August of 2009 to May of 2013, where she completed her Bachelor of Science with a major in Biology and a minor in Chemistry. Following graduation, she continued her graduate studies at ASU from January of 2014 to May 2016 and completed her Master of Science degree in Biology. Her major field of specialization was parasitology, where she developed skills in animal necropsy procedures and in handling and preparation of parasite specimens. Additionally, she acquired experience in performing molecular techniques and procedures such as DNA extraction, PCR amplification, gel electrophoresis, PCR purification, and sequence analysis. Gizell Vasquez's permanent address is 506 W American Blvd. in Muleshoe, TX 79347.